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FEEDING AND PHARMACOLOGICAL PROPERTIES OF *ANISODORIS NOBILIS* INDIVIDUALS (MOLLUSCA; NUDIBRANCHIA) SELECTING DIFFERENT NOXIOUS SPONGES PLUS DETRITUS

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ABSTRACT

Individual differences in *Anisodoris nobilis* food selection were sought in the natural environment, to determine why their digestive glands differed pharmacologically. Of 73 stationary *A. nobilis* specimens turned over in the initial survey, 84% of those on sponges and 65% of those on detritus had their mouths everted; no others were feeding. Microacoustic observations showed that an individual stops on a sponge, everts its proboscis for a long period, then rasps the sponge. Field studies of individual diets were made without disturbing the animals, by tabulating the foods on which each nudibranch was found to be stationary.

Twelve individual nudibranchs observed subtidally about three times daily for 8 days differed significantly in the sponge species they selected. Each individual was found stopped on detritus during roughly 50% of the observations, remaining there for ~1 day at a time. Each nudibranch stopped for <2 days on a sponge, sometimes returning to that spot during subsequent days. Among individuals transplanted to different feeding sites, each everted its mouth only when on the sponge species it had been eating at the previous site. Two months of subsequent, scattered observations showed occasional longer-term feeding differences.

Noxious histamine (present in each sponge) and doridosine (found only in the nudibranch) accounted for different pharmacological properties in bioassays of *Anisodoris* digestive gland extracts. Histamine disappeared when the nudibranchs ate detritus, which suggests that this mixed diet purges sponge compounds from the nudibranch. Extracts from edible sponges administered to nudibranchs caused fleeing, while extracts of avoided sponges caused violent contractions, increased mucus production, and even death.

INTRODUCTION

Studies on food selection and other resource utilization usually deal with pooled data on groups of individual organisms. However, adjacent and apparently similar members of the same species may select different diets in "intraspecific resource partitioning" (Kitting, 1979). Roughgarden (1972) considers theoretical aspects of conspecific individuals choosing different foods from a "continuous" resource axis. The present study uses graphic methods to analyze the behavior of individuals in relation to certain discrete resources that have pharmacological effects on the animal.

The dorid nudibranch *Anisodoris nobilis* (MacFarland, 1905) has biologically potent compounds concentrated in the digestive gland (Fuhrman *et al.*, 1980). This and other species of dorid nudibranchs feed largely on sponges (Harris, 1973),

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which themselves often contain unusual noxious compounds (*e.g.* Ciereszko, 1970; Bakus and Green, 1977; Minale, 1978). The tropical nudibranch *Phyllidia varicosa* concentrates terpenoid compounds from sponges (see Burreson *et al.*, 1975). Some anaspidean opisthobranchs, relatives of the nudibranchs, also modify such compounds (Faulkner and Ireland, 1977), and perhaps some nudibranchs do this too.

Initial pharmacological studies on *Anisodoris nobilis* from the Monterey Bay area showed that extracts of different groups of individuals had different effects on rate and amplitude of contractions of mammalian atria and smooth muscle (Fuhrman *et al.*, 1979). The present study shows that these differences in pharmacological properties reflect sponges or detritus eaten recently by individual nudibranchs, which differ in the sponges they select. The study also illustrates how a consumer's foraging patterns may minimize harmful effects of noxious foods.

MATERIALS AND METHODS

Field experiments were carried out 100 m offshore from Hopkins Marine Station (36°37′N; 121°54′W) at the south end of Monterey Bay, California. The study area is a rocky subtidal habitat near 10 m depth within a large stand of giant kelp, *Macrocystis pyrifera. Anisodoris nobilis* adults measuring about 3–6 cm in length were common on the sides of large rocks up to 4 m in diameter. These rocks were separated by coarse sand. Individual nudibranchs were generally several meters apart, slow-moving, of different sizes, and with individually distinctive color markings. Therefore, I was able to recognize and observe specific individuals for over a week without artificial marking. Periodically, underwater photographs of each individual were made to confirm its identity.

Sponges eaten by the nudibranchs encrusted the substratum in thin sheets. Nudibranch feces were collected *in situ* with eye droppers, and samples of spicules were taken from sponges for use in identification. To assess nudibranch diets in the natural environment without disturbing the animals, I recorded the foods on which nudibranchs were observed to be stationary. I could rarely see if the nudibranchs were eating unless I turned the animals over, but in initial observations, 26 of the 31 *Anisodoris nobilis* found stationary on sponges were feeding (see below). Often, sponge had been excavated beneath the mouth of a stationary individual. Nudibranchs moving over sponges or detritus were never found feeding. Defecation was rarely seen, but the few successful fecal collections confirmed that the sponge species under an everted mouth had been consumed. Therefore, tabulations of foods under stationary individuals reflected their diets. Attempts to conduct feeding experiments in the laboratory were generally unsuccessful. Nudibranchs kept in aquaria with rocks bearing sponges moved about a great deal and seldom fed.

To determine the diets of *Anisodoris nobilis* individuals, I followed 12 nudibranchs on three large subtidal rocks abundantly supplied with the four types of food most commonly eaten. About 3 dives were made each day for 8 successive days during late September 1978, usually at 0800, 1200, and 1600 h. During each dive, I mapped each individual's location, measured the distance from the point where it was last seen, and noted any food on which it was sitting if the animal was stationary. From early October through February 1979, further detailed observations in the study area supplemented this routine sampling. Statistical methods used on the data appear in Sokal and Rohlf (1969).

Sponges and nudibranchs used for chemical assays were collected adjacent to the primary study area. Chemical extractions and sensitive pharmacological bioassays in which I merely assisted were carried out by Prof. Frederick Fuhrman and

Mrs. Geraldine Fuhrman of the Department of Physiology, Stanford Medical School. The assay methods, outlined in Fuhrman *et al.* (1979) and Fuhrman *et al.* (1980) make use of the spontaneously beating guinea pig heart *in vitro* (Perry, 1968) to detect concentrations of compounds such as ~0.4 μg histamine, or ~15 μg adenosine, per gram of nudibranch, sponge, or other tissues (Chang *et al.*, 1978). Logarithmic increases in dose were necessary to cause linear changes in response (Fuhrman, unpublished data).

I also conducted a bioassay on nudibranchs to detect toxic effects of extracts of various foods on them. An extract of each food (a sponge species or detritus from the habitat) was prepared by grinding 0.50 g of fresh sample in 1.0 ml of filtered seawater in a mortar. The toxic effects were assessed by observing changes in nudibranch movement and heart rate after extracts were applied. I timed sequences of heart beats by observing the heart's shadow with a 7× microscope. A focused, 7.5-volt light source illuminated each nudibranch from below its clear bowl of seawater. Only nudibranchs less than 4 cm in length were thin enough to use.

Some extracts were administered externally to nudibranchs, to approximate natural exposure of feeding animals to exudates of macerated food. Doses consisting of 0.05-ml samples of extract were perfused near the heart and gills of individuals 2–4 cm long, which were kept in 200-ml bowls of clean seawater at 12.5 ± 0.25°C. A solution of serotonin (10^{-5} *M* in filtered seawater) was used for comparison. Serotonin (5-hydroxytryptamine) is a molluscan heart neurotransmitter known to increase the rate of contraction of clam heart if applied directly to the heart at 10^{-6} *M* (Greenberg *et al.*, 1973). Filtered seawater was applied similarly as a control.

Other extracts were applied internally to nudibranch hearts *in vivo.* These extracts were diluted 1:10 with filtered seawater before use, and were injected into the pericardium. Nudibranchs 4 cm long received 0.2-ml doses and those 2 cm long received 0.05 ml, the doses representing roughly 2% of the digestive gland volume. Again, serotonin (10^{-6} *M* in this case) and filtered seawater were used for comparison. To test for the generality of the results obtained with *Anisodoris*, I also administered extracts to the more common dorid nudibranch *Doriopsilla albopunctata.*

RESULTS

Foraging behavior

In the laboratory, when *Anisodoris* stopped on a sponge, the animal remained in place for up to ~2 days. Whenever an individual remained stationary with its proboscis visible under its dome-like body, or when stationary animals were lifted slightly at intervals, the mouth region was found to be everted for long periods. Underwater microacoustic observations with a contact microphone (Meshna, Inc., P.O. Box 62, E. Lynn, Mass. 01904; also see Kitting, 1979) placed on sponges showed that radular scraping occurred less than 1% of the time in either light or darkness, even when the proboscis was everted. Sponge tissue under the nudibranch's mouth appeared partially dissolved even before it was seen or heard to be rasped, as though external digestion occurred first. The rasping that finally occurred was gentle brushing and irregular picking at the sponge, which is packed with siliceous spicules about 0.3 mm in length. Each nudibranch excavated a cavity up to about one-fifth its own body size in a sponge in about 2 days. Since this feeding involved eversion of the mouth followed by occasional rasping, eversion of the mouth was interpreted as "feeding behavior" in the observations below.

Overall, *Anisodoris* was found with its mouth everted on the following foods, listed in order of decreasing frequency: finely divided detritus adhering to the surface of encrusting algae or open rock; an orange sponge, *Axocielita* (=*Esperiopsis*) *originalis*; a crimson sponge, *Antho* (=*Isociona*) *lithophoenix*; a gray sponge, *Astylinifer arntdi*, growing on heavy lower blades of kelp, *Cystoseira osmundacea*; and a blue sponge, *Hymenamphiastra cyanocrypta.* Actual feeding damage was observed on each of the sponges. Additionally, fecal analyses showed that *Anisodoris* also ate a less common sponge, *Myxilla parasitica.* When *Anisodoris* was examined during 98 initial daytime field observations, the mouth was everted in 26 of the 31 animals stopped on sponges, in the 26 of the 40 stopped on detritus, but in none of the 25 animals traveling. The remaining two animals were stopped on clean encrusting algae, and were not feeding. Supplementary diving and laboratory observations made at night showed no noticeable differences in *Anisodoris* behavior.

Observations on the 12 or more *A. nobilis* specimens within a 100 m^2 area examined thrice daily for 8 successive days are summarized in Table I. Individual nudibranchs often remained stationary for several successive observations. The animals averaged only 9 cm net displacement between successive observations, but even this limited movement made all four common foods readily accessible to all individuals. Typically, after traveling for about a day, an individual appeared to remain in the same location and orientation on a sponge for up to about 2 days, usually with its mouth everted. Then the site would be found vacant, with a small wound in the sponge, while the nudibranch was found up to 2 m away, eating detritus or crawling. During at least 6 of 20 instances in which animals traveled but were found consistently, the individual returned to within <10 cm of its old feeding site (e.g., Fig. 1). One individual returned 2 m to its previous feeding site within 4 h. Excavated areas of sponge appeared to regenerate quickly (Fig. 1), and in no instance was a patch of sponge found to disappear with repeated feeding.

Differences in the foods on which individuals stopped

The foods used by each individual repeatedly examined in the field are compared in Table I. The only food omitted is the very sparse detritus on algae. No individual nudibranch was found on every dive, and several foraging records ended because the individuals were collected. On the average, each animal was observed stationary on food 7–8 different times. A *G* test showed highly significant differences among the sets of foods that different individuals occupied ($G = 121.19$; 33 df; $P \ll 0.005$). Similar differences were detected for those six animals found stationary on food in at least five observations throughout the study ($G = 36.92$; 10 df; $P < 0.005$). To help ensure independence of observations from one sampling time to the next, data taken closest to noon each day (for each individual) were also tested. This yielded a similar outcome ($G = 49.28$; 16 df; $P < 0.005$).

Since complete sequences of observations were not available for each individual, it is possible that on occasions when they were *not* seen they were away eating other foods. Therefore the data were also supplemented to bias them in favor of the null hypothesis (that foods used were independent of the individual's identity); hypothetical data were added as though each individual ate an additional food each time it disappeared and then reappeared during the sequence of observations. Even

TABLE I

Foraging records of adjacent Anisodoris nobilis *individuals on detritus* (=De), *encrusting noncalcareous algae* (=En), *crustose coralline algae* (=Cr), *erect algae* (=Er), *red* Axocielita (=R), *blue* Hymenamphiastra (=B), *gray* Astylinifer (=G), *or traveling* (=→). Dot = not observed; ? = food could not be determined.

	Date for sampling times:									Material at anterior if nudibranch was stationary								
Animal	9/25			9/26			9/27			9/28		9/29		9/30			10/1	10/2
A	→	De	·	·	→	·	·	R	De	R	G	·	·	R	R	De	R	Cr
B	De	R	R	Er	·	·	·	De	R	R	R	R	R	R	De	R	De	Cr
F	De	De	En	En	→	En	·	·	·	·	·	·	·	·	·	·	·	·
I	De	·	De	·	De	De	·	B	·	·	·	R	·	·	·	·	·	·
J	·	·	En	De	R	R	De	De	→	En	·	·	·	·	·	·	·	·
M	De	De	→	·	R	De	→	→	En	Er	R	R	R	R	De	De	R	·
Q	·	·	→	→	→	·	→	→	→	→	→	→	→	En	→	De	→	De
R	→	·	·	?	R	·	R	R	R	R	R	·	R	R	R	R	R	R
S	B	B	B	B	B	·	B	·	B	B	B	En	·	B	B	B	B	B
W	·	·	R	·	·	·	En	?	De	·	·	De	→	·	·	·	·	·
Z	·	·	Er	·	·	·	·	·	·	R	R	De	·	→	De	→	R	R
ZZ	·	→	→	→	En	→	?	?	De	De	Er	Er	En	De	·	·	De	·

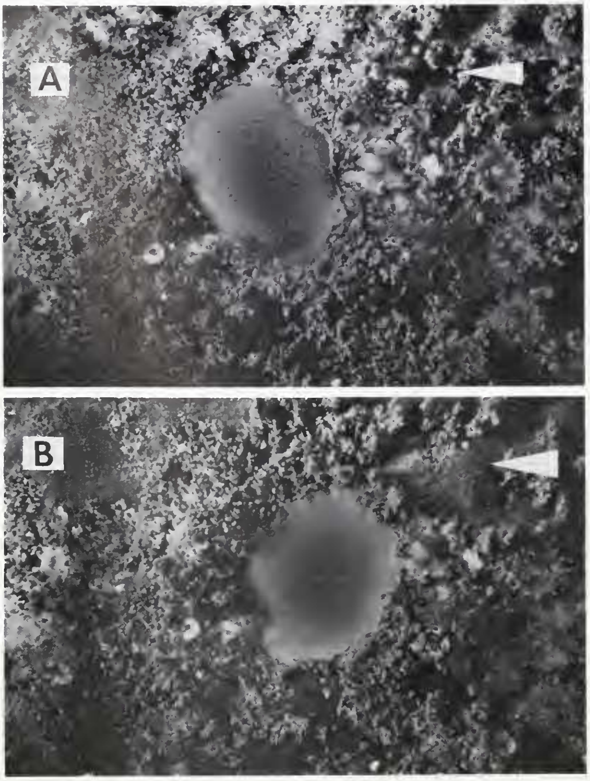

FIGURE 1. *Anisodoris nobilis* "Individual R" measuring 20 mm, down in an irregularity among its sponge food, *Antho lithophoenix*, photographed 5.5 weeks apart in the same place (also suitable for stereo viewing). (A) December 4, 1978. Retracted rhinophores are positioned toward bottom of photograph. Extensive feeding damage since October 25 is evident as upper left and upper right dark cavities (see arrow). (B) January 12, 1979. Retracted rhinophores are toward top of photograph. Feeding damage has regenerated extensively in upper left, and partially in upper right (see arrow).

under this conjectural condition, the differences among individuals remained highly significant ($G = 143.49$; 44 df; $P \ll 0.005$).

Applying data on individual foraging to a graphic model developed by Kitting (1978) enabled more detailed analysis of differences in discrete resources used by individuals. Figure 2 illustrates the frequency distributions of individual diets where the percentage of times the individual was observed to be stationary on each food resource defines its "diet" (as a resource use). Hypothetical frequency distributions were calculated from binomial expansions of the average dietary percentage represented by each food, for all individuals combined. The formula for the plot is a binomial expansion,

$$(b + a)^n = \sum_{i=O}^{n} \binom{n}{i} a^{n-i} b^i$$

where a is the average percentage of feeding on a particular food, b is the percentage of feeding on other foods (=1 − a), n is the average number of observations (about seven) in which an individual was found stationary on food, and the coefficient

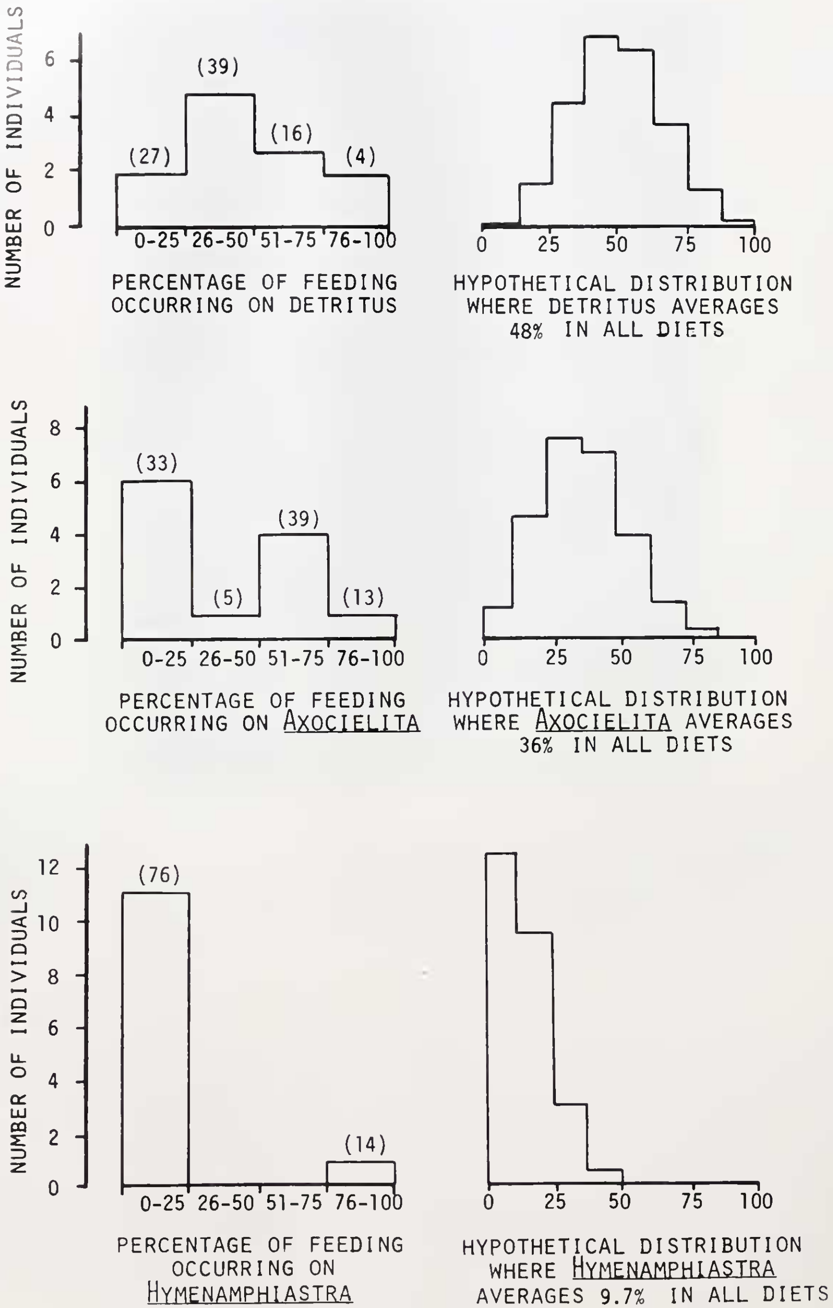

FIGURE 2. Frequency distributions of the numbers of individuals observed to be stationary (and probably feeding) on each food at various frequencies. Parentheses enclose numbers of observations. Kolmogorov-Smirnov test for goodness of fit between each observed and hypothetical distribution ($N = 90$): detritus $D = 0.105$, $P > 0.20$. *Axocielita* $D = 0.276$, $P < 0.05$. *Hymenamphiastra* $D = 0.428$, $P < 0.01$. *Astylinifer* (eaten only once in this experiment) is not analyzed.

term with n and i is each constant given by Pascal's triangle. Each product in the overall sum represents merely the expected probability of sampling an individual on a particular food (n − i) times during n observations (Kitting, 1980). If some individuals were to yield very few feeding occurrences and increase the variance of the actual distribution, one could sum hypothetical distributions, each for a single individual's n (the number of times it was observed stationary on food).

Comparing the hypothetical distribution to the actual numbers of individuals observed on each food (Fig. 2) shows that individuals tended to be stationary on detritus about 50% of the time. The detailed observations made over the 8-day period showed that detritus was eaten at intervals between feeding on a given sponge species (Table I). Furthermore, each individual was stationary on *Axocielita* either most of the time or very rarely. Only one individual appeared to feed on *Hymenamphiastra* in this experiment, and this nudibranch was never observed to eat any other sponge.

To confirm that different individuals tended to choose particular sponge species, even when alternative sponges were readily accessible, "transplant" experiments were conducted. Numerous nudibranchs were lifted carefully from *Axocielita, Astylinifer, Hymenamphiastra, Antho*, or detritus. If the mouth had been everted, the animal was placed (within 30 s) in a location where another individual had been feeding in previous observations. If individuals all had the same food preferences, those foods hypothetically most preferred would be eaten without regard to what the individual had been eating. However, individuals placed on another individual's feeding site or other sponges continued feeding only if the type of food remained the same; in 20 trials involving numerous individuals moved to another source of the same food, only six showed no mouth eversion (three to five trials for each of five foods). In contrast, individuals transferred to a different type of food rejected the other food in all 49 such trails (one trial for *Astylinifer* to *Antho*, one for *Antho* to *Astylinifer*, and two to five trials for each of 18 other combinations).

In a related test, two crawling (not feeding) individuals were placed successively on each of the same five foods, and allowed 5 min on each. One animal everted its mouth only when placed on *Hymenamphiastra*, and did this repeatedly, as though it had been seeking that food. The other crawling animal rejected each of the foods repeatedly. Although individuals differed somewhat in size, shade of yellow, and density of black markings, such differences showed no consistent relationship to the food preferred.

After the initial 8 days of routine sampling, most identified individuals could no longer be located. However, two nudibranchs were observed eating only *Hymenamphiastra* and detritus intermittently over 2 months. During the same 2 months, 2 of 10 individuals on specific rocks in sand were observed eating the sponge *Antho*, yet none of the nudibranchs on those rocks had eaten *Antho* during the earlier 8 days of the observations (see Animal R; Table I and Fig. 1).

In the laboratory tests, all individuals began eating *Astylinifer* within 1 week, even when other food sponges were present. However, no individuals were ever observed eating *Tethya aurantia* var. *californiana, Stelletta clarella*, or *Polymastia pachymastia*, although these species were among the most common sponges in the study areas. In the field, food species occurred in the following order of decreasing abundance: *Hymenamphiastra* > *Axocielita* > *Antho* > *Astylinifer* > *Myxilla*. The number of individuals observed eating each species during two months yielded the order: *Axocielita* > *Antho* > *Astylinifer* > *Hymenamphiastra* > *Myxilla*. Spearman's rank correlation coefficient showed no significant overall difference here

TABLE II

Bioassays of Anisodoris nobilis *digestive gland extracts. Ultrafiltrates were added to spontaneously beating guinea pig atria* in vitro. *Corresponding chemical analyses of histamine (after Shore, 1971) are noted where available. Decreases in heart action are in boldface type. Hist. = histamine.*

Description (and number of individuals)		Mean (and range) of mg extract in 8-ml bath	Heart action as mean (or range) of % initial action:	
			Amplitude	Rate
Fresh, unstarved *Anisodoris* feeding on:				
Axocielita	(3)	55 (33–66)	(133–180)	(100–130)
Astylinifer	(2)	90 (80–100) (with 0.4 μg hist./g)	(112–120)	(100–112)
Hymenamphistra	(1)	100 (with 0.7 μg hist./g)	151	148
Antho	(2)	67 (33–100) (with 0.5 μg hist./g)	(100–155)	(100–147)
Antho	(1)	133 (with 0.7 μg hist./g)	**54**	**70**
Myxilla	(1)	66	150	150
Detritus	(3)	93 (80–100)	**(55–80)**	**(50–100)**
Anisodoris starved 4 days (mean of 1–2 individuals from each of five sponge species)		65 (30–100)	135 (122–148)	114 (100–140)
Anisodoris starved 10–20 days (mean of 2–3 individuals from each of three sponge species)		67 (33–130)	**84 (60–115)**	**81 (61–100)**
Histamine standards		(0.050 μg hist./dose)	123	irregular
		(0.300 μg hist./dose)	169	irregular

($r_s = 0.60$; 3 df; $P \approx 0.5$), since the nudibranch population ate these particular sponges in roughly their order of abundance, with the exception of the rarely eaten, common *Hymenamphiastra.*

Pharmacological properties of individual nudibranchs and foods

In pharmacological assays, the extract ultrafiltrates ranged from strongly excitatory to strongly depressant in their effects on contraction rate and amplitude of guinea pig heart. Table II compares results of assays using extracts of digestive glands of *Anisodoris* individuals. Differences among nudibranchs did not correspond to the sponge species being eaten at the time of collection. Extracts of individuals eating each sponge species caused some increase in rate and amplitude of the guinea pig heart in the assay, except for one seemingly normal individual eating *Antho.* This nudibranch and all three collected as they ate detritus produced a decreased rate and amplitude in the heart assay.

When *Anisodoris* specimens freshly collected as they ate sponges were starved for 2 days (half the period over which *Anisodoris* continued to defecate), ultrafiltrates of their digestive glands still yielded excitatory effects in the guinea pig heart assay. However, when such individuals had been starved 10–20 days, their digestive gland ultrafiltrates produced decreased heart rate and amplitude in the assay (Table II). As expected, analogous tests with feces themselves showed an excitatory effect in the heart assay.

TABLE III

Sponge extract ultrafiltrates assayed with guinea pig atria. Histamine (hist.) analyses are noted where available. Mean increases in heart action greater than the 130% average overall effect are in boldface type.

	Sponge (and number of replicates)		Mean (and range) of mg extract in 8-ml bath	Heart action as mean percent (and range) of % initial action:	
				Amplitude	Rate
Eaten species	*Axocielita*	(4)	154 (44–220) (with 0.9 μg hist./g)	127 (107–153)	120 (100–138)
	Astylinifer	(2)	64 (27–100) (with 0.2 μg hist./g)	**139** (132–145)	115 (110–119)
	Hymenamphiastra	(4)	107 (27–220)	121 (100–157)	114 (100–120)
	Antho	(4)	110 (66–165)	108 (100–120)	96 (85–110)
	Myxilla	(1)	133 (with 0.2 μg hist./g)	125	**150**
Avoided species	*Stelletta*	(6)	95 (25–200)	**171** (146–187)	**137** (123–155)
	"(outer)		(200)	(187)	(142)
	"(inner)		(140)	(177)	(123)
	Tethya	(2)	88 (50–125) (with 0.3 μg hist./g)	**149** (135–162)	127 (125–128)
	Polymastia	(3)	133 (50–250)	**156** (113–188)	— (irreg.–128)
	Mean of eaten species		116	124	119
	Mean of avoided species		104	**159**	**132**

Table III summarizes analogous assays using ultrafiltrates of extracts of various food sponges plus similar extracts of three sponge species that *Anisodoris* avoided. All sponge species showed an excitatory effect in the guinea pig heart assay, although the effect of the sponge *Antho*, an acceptable food for *Anisodoris*, was very slight. Replicate experiments on single sponge colonies were not consistent enough to enable detailed comparisons between separate sponge colonies, but no major differences in results were evident among colonies of given sponge species. Overall, the three sponge species avoided by *Anisodoris* yielded 30% higher amplitudes and 10% higher rates in the heart assay, relative to the results from similar doses of readily eaten sponge species (Table III). Distinct outer and inner tissues of the avoided sponge *Stelletta* both caused roughly the same increase in the assay. Analogous tests with detrital food available to the nudibranchs produced no effect in the assay.

Histamine, which increases contraction rate and amplitude in the mammalian heart assay, was detected in extracts from sponges and *Anisodoris* digestive glands (Tables II and III). Histamine was present in eaten and avoided sponge species. Generally, enough histamine was present to account for excitatory effects of ultrafiltrates of both fresh *Anisodoris* and sponges in the heart assay. However, the amount of histamine did not parallel the strength of excitatory effect in the heart assay; apparently, other compounds also affected assayed heart action.

A methyl purine riboside named doridosine (N^1-methylisoguanosine) extracted from *Anisodoris* digestive gland strongly decreases contraction rate and amplitude of guinea pig atria (Fuhrman *et al.*, 1980). To determine if the compound could have been obtained from the nudibranch's food, sponge species eaten by *Anisodoris* were extracted and analyzed by the same methods. However, bioassays and thin-layer chromatography of column eluates revealed no doridosine or adenosine in any sponges tested (Table III). Fuhrman (personal communication) calculates that the sponges, if they contain the compound, have less than 1.6–2.2 μg purine riboside (as adenosine) per gram wet weight. This would be < 1% of the concentration in the nudibranch digestive gland.

Thus, the digestive gland extracts from starved nudibranchs, and from nudibranchs recently eating detritus, decreased rate and amplitude of muscle contraction in mammalian heart bioassays; this is a characteristic effect of doridosine. However, extracts from sponges showed an excitatory effect, characteristic of histamine detected in sponges.

Effects of food extracts on nudibranchs

In the field, nudibranchs periodically leave their feeding sites on sponges. It seemed possible that sponge exudates released during nudibranch feeding might exert some gross influence on the nudibranchs and stimulate them to leave. To test this, unfractionated sponge extract was applied to the animals externally, roughly simulating natural exposure. Three sponges were tested: the readily eaten *Axocielita*, the seldom eaten *Hymenamphiastra*, and the avoided species *Tethya*. Extracts were applied externally to two specimens of *Anisodoris* and two of *Doriopsilla*. Thirteen trials were made with each sponge extract. Seawater alone was applied as a control. Nudibranch heart rates and behaviors were then monitored 5–7 times per trial.

In these experiments, heart rates before and after each application averaged 44 ± 3 (SD) beats per 30-s observation. Analogous tests on the dendronotacean nudibranch *Dendronotus albus*, which does not eat sponges, also showed no change in heart rate. However, overt behavior of all the nudibranchs was clearly affected in the experiments. The application of each sponge extract was followed by each dorid or *Dendronotus* specimen retracting its appendages and moving away. The application of seawater as a control yielded no response. Applications of *Anisodoris* digestive gland extract produced clear movement away from the extract, but still did affect nudibranch heart rate.

Two analogous tests with *Anisodoris* bathed in 10^{-5} M serotonin suggested that such low-molecular-weight amine compounds produce little internal change when applied externally to the heart and gill region; heart rates remained near 44 ± 3 (SD) beats per 30 s upon applying serotonin externally, unlike serotonin's marked effect on clam heart (see Greenberg *et al.*, 1973). In marked contrast, external application of serotonin to nudibranchs caused marked external changes, including prolonged retraction of appendages and copious secretion of mucus from the animal's dorsal surface.

To test for toxicities of available foods, extracts of the sponges *Axocielita* and *Tethya* and of detritus available as food in the nudibranch habitat were prepared as described earlier. These were diluted 1:10 in seawater and injected into the nudibranch's pericardium *in vivo*. Each sponge or detritus extract was injected into two *Anisodoris* and two *Doriopsilla* specimens. Control animals (four *Anisodoris* and six *Doriopsilla* specimens) received only seawater injections. After injections of food extract, these animals were maintained in a 1:100 dilution of extract in seawater; this helped compensate for injected extract diffusing out of the nudibranch, and simulated internal plus external exposure to exudates of wounded sponge (or macerated detritus).

All injections, of sponge, detritus, or seawater only, produced temporary overall body contractions. No obvious change in heart rate occurred after seawater injections. Rates before and after injection averaged with ±3 beats (SD) of the initial 44 beats per 30-s observation (repeated 4–5 times for each individual). Detritus extracts likewise produced no significant change. In both nudibranch species injected with sponge, the most frequently eaten sponge (*Axocielita*) as well as the most conspicuously avoided sponge (*Tethya*) initially halted nudibranch heart action, although the nudibranchs continued to move about. The heart of *Anisodoris* began beating again intermittently after roughly 1 h. *Tethya* halted *Doriopsilla* heartbeat for over 12 h, after which heartbeats were intermittent. In both nudibranch species, *Tethya* injections produced thick mucus and violent body contractions. Serotonin (at 10^{-5} M in seawater, similarly injected into five specimens of *Anisodoris* and 5 *Doriopsilla*) temporarily halted heart action or produced no clear change. Only *Tethya*-injected nudibranchs died (two *Anisodoris* and two *Doriopsilla* specimens). Other individuals injected with food or serotonin appeared healthy while they were maintained in seawater for over 1 week following the experiments.

In summary, none of the sponge extracts showed marked effects on *Anisodoris* or *Doriopsilla* heart rate *in vivo* when applied externally, but all repelled the nudibranchs. When injected into *Anisodoris*, extracts of eaten and uneaten sponge species led to halted, then irregular heart beating, while avoided *Tethya* eventually killed *Anisodoris* and *Doriopsilla*.

Discussion

Anisodoris nobilis was the most frequent predator on sponges observed in the present study. It fed on at least five sponge species representing two families in the order Poecilosclerida. This nudibranch conspicuously avoided more than three common sponge species representing two other orders. *A. nobilis* at different localities appears to eat other sponge species (see McDonald and Nybakken, 1978) not common in the present study locality. Foraging differences observed among individuals in the present study did not correspond to differences in physical appearance or sex (all animals observed were mature and hermaphroditic). However, observations beyond the 8-day experiment show that *Anisodoris* individuals sometimes change their food species. The nudibranchs' different shades of color may possibly result from long-term dietary differences.

A striking finding was that different *Anisodoris nobilis* individuals tended to choose different sponge species, even though the nudibranchs traveled among the same set of available sponges for over 1 week. Analogous differences in the foods selected by conspecific individuals sharing the same food resources have been noted. Gain (1891), discussing the diets of land snails, remarked "perhaps individualism may account for some of the variation." Documented examples among invertebrates include work on insects reared on different plant species (reviewed by Hanson, 1976) and studies on bee foraging (reviewed by von Frisch, 1961, and Heinrich, 1976). It appears that such foraging differences among adjacent conspecific individuals may account for differences in natural, diet-dependent carbon isotope ratios of individual grasshoppers (Fry *et al.*, 1978). For marine invertebrates maintained in the laboratory, Landenberger (1968) noted that one *Pisaster giganteus* sea star consistently selected snails rather than the usual mussels as food, and Paine (1963) observed that one cephalaspid opisthobranch *Navanax* (*Chelidonura, Navarchus*) *inermis* consistently ate certain nudibranch species that all other individual *N. inermis* rejected. Studying carnivorous snails in the natural environment, Lani West (unpublished manuscript, Hopkins Marine Station) documented marked dietary differences between individual snails (*Nucella emerginata*) exposed to the same mussels, limpets, and barnacles.

Additional studies on more species are needed to determine how widespread such intraspecific resource partitioning is. It is tempting to account for the phenomenon itself by considering it analogous to the more familiar interspecific competition and resource partitioning (see Lawlor, 1980). In general, past interference competition (*e.g.* aggression) might have excluded neighboring individuals from a given animal's chosen resources, and favored evolution of such intraspecific resource partitioning.

However, no suggestion of interference or exploitative competition appears among these nudibranchs. Their intraspecific resource partitioning could be a simple consequence of several resources being available to the population, while an individual's specialization may be advantageous for locating, consuming, detoxifying, or digesting a distinctive food (Kitting, 1980).

Studies on diet mixing by individual limpets (Kitting, 1980) suggest that intraspecific resource partitioning may be uncommon among animals that avoid excessive amounts of given foods. The present results show that while individuals use some different food resources, they may avoid excessive, repulsive amounts of a single type of food by sharing detritus.

In the present study, the nudibranch's ability to relocate a particular feeding site after extended absence was partly responsible for observed differences in individual diets. It is unknown how these nudibranchs, or certain limpets that return to foraging sites (Kitting, 1978) and home sites (*e.g.* Breen, 1971) navigate back to previous sites.

Though the mammalian heart assay showed differences in chemical constituents among *Anisodoris* individuals, the differences did not correspond to the sponge species eaten. Instead, the unusual decreases in rate and amplitude of heart beat in the bioassay are associated with recent consumption of detritus, and with starvation in the laboratory. Apparently, varying amounts of histamine in the sponges (and in the digestive glands of fresh *Anisodoris*) mask the distinctive assayed effect of doridosine in the nudibranch's digestive gland. Analogous assays of *Anisodoris* extracts made from nudibranchs collected on different species of sponges at Santa Barbara, California, and at San Juan Island, Washington, suggest that this combination of doridosine and sponge histamine occurs in the nudibranchs over much of the geographic range of *Anisodoris* (Furhman, Fuhrman, and Kitting, unpublished data). Since no doridosine or another purine riboside, adenosine, was detected in the available sponges, the nudibranch appears to produce the compound or concentrate it over 100× from undetected trace amounts.

An Australian sponge, *Tedania*, is reported to contain doridosine in unspecified but extractable concentration (Gregson *et al.*, 1979). *Tedania* is in the same family as several sponges eaten in the present study: *Astylinifer, Hymenamphiastra*, and *Myxilla*. If individual nudibranchs or other animals each took up different compounds from different foods, each individual might benefit from the diverse collection of derived chemical defenses against higher-order consumers. Other, externally secreted compounds appear to be common defenses for nudibranchs (*e.g.* Thompson, 1976); fishes learn to avoid such colorful, distasteful objects as *Anisodoris nobilis* (see Kellogg, 1980). The defensive functions, if any, of internal compounds such as doridosine itself are not yet clear.

The bioassayed differences found among nudibranch digestive glands are associated with the animals' content of sponge histamine. After *Anisodoris* ceased feeding on sponge temporarily and ate detritus (apparently for less than a day), histamine was not detectable in the digestive gland. The same result appeared when *Anisodoris* had not eaten for 8 days. Presumably, other compounds from sponges also could be purged or destroyed relatively quickly during periodic feeding on detritus. The results of applying sponge extracts to *Anisodoris* suggested that nudibranchs are somewhat resistant to compounds in extracts of edible sponges, though they are not completely tolerant of sponge compounds.

In the mammalian heart assay, unpalatable sponges were somewhat more biologically active than edible sponges were, but one avoided sponge eventually killed *Anisodoris* and *Doriopsilla.* Moving away and eating detritus periodically could be advantageous to nudibranchs in preventing sustained, high external and internal levels of sponge constituents such as histamine. Furthermore, a nudibranch's rasping on sponge tissue seems by audible evidence infrequent, slow, and laborious (also see Forrest, 1953); periodic feeding on detritus might also be advantageous in preventing excessive exposure to sponge spicules.

Chemical findings suggested the use of *Anisodoris nobilis* for the present studies on intraspecific variation in chemistry and feeding. Pharmacological assays with a mammalian heart documented basic chemical differences among *Anisodoris* individuals recently eating sponges or detritus. Unusual pharmacological effects of doridosine in nudibranch digestive gland were the opposite of effects of major biologically potent compounds in the sponges, including histamine. Further chemical investigations may find even more potent compounds in unpalatable organisms such as sponges, and may find antidotes or antagonistic compounds in specialist predators (individuals or species) that eat these unpalatable organisms. However, a specialist predator's resistance to its chemically defended foods need not be complete, if additional foods such as detritus often purge noxious compounds from a predator's digestive system.

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